

**THE MALPIGHIAN TUBULES OF Aedes Aegypti L.
(DIPTERA, CULICIDAE).**

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This paper reports observations dealing with (1) the gross structure of the Malpighian tubules of *A. aegypti* in all instars during development and (2) the histological structure of the tubules of the fourth larval instar.

METHOD.

The gut and attached tubules were dissected from the body in a physiological saline medium and examined in the living condition or fixed for further study as whole mounts or sections. Whole mounts were prepared by using Buxton's medium or by fixing in osmic acid vapor followed by staining in methyl green. Material to be sectioned was fixed in Carnoy's or Bouin's fluid and stained with iron haematoxylin or Mallory's triple stain. Golgi bodies were demonstrated by fixing the tubules in 3% potassium dichromate and 1% osmic acid (3:1) followed by treatment in 3.5% potassium dichromate (2-5 days) and 1% silver nitrate (1-2 days). All sections were cut at 6 micra. The basement membrane was demonstrated by using 0.01 normal sodium hydroxide.¹

OBSERVATIONS.

There are five Malpighian tubules which arise just anterior to the constriction between the stomach and intestine. Four of these arise laterally, two on each side, while the fifth arises dorsally. The arrangement of the tubules around the posterior stomach region is such that the distance between the two more ventral tubules is about twice that of the distance between any two of the others. Each tubule extends anteriorly to the mid-stomach region, then turns sharply and extends posteriorly to the rectum. Some crossing of the tubules occurs in their posterior course. The blind ends of the tubules are attached to each other and to the rectum by means of tracheoles; elsewhere along their course the tubules are similarly attached to each other but are free of any connection to the gut or body wall.

¹ Wigglesworth, V. B. 1939. The principles of insect physiology. E. P. Dutton and Co., New York. Contains excellent bibliography.

In freshly dissected tissue the tubules are white, translucent structures consisting of large cells set upon a transparent basement membrane. The nuclei of these cells are visible as clear areas within the cytoplasm. The outer limits of the cells can be readily observed through the basement membrane. In the first three larval instars the inner limits of the cells may be observed along the lumen of the tubules as a layer with a different refractive index. In the fourth larval instar and in the pupa and the adult the inner limits of the cells can be observed only in the proximal regions of the tubules. Most of the cells of the tubules are triangular with their apices projecting into the lumen giving to it a tortuous appearance. For a short distance close to the entrance of the tubule into the gut the cells are of the flattened cuboidal type and the lumen appears as a straight tube.

In all instars except the first the tubules increase in size distally, the diameter of the blind end being about two times greater than that at the point of origin. This increase in tubule size is accomplished by an increase in cell size rather than in cell number. During development the tubules increase rapidly in size from one larval instar to the next, those of the mature larva being approximately eight times longer than those of the first instar larva. The tubules of the pupa and adult are slightly smaller than those of the mature larva. The variations in tubule size from proximal (minimum) to distal (maximum) ends and from one instar to another are indicated (Table 1).

TABLE 1. Size of the Malpighian tubules of *Aedes aegypti* L. (all measurements in millimeters).

	Larval instars				Pupa	Adult
	1	2	3	4		
Diameter :						
Minimum	0.017	0.017	0.035	0.072	0.064	0.060
Maximum . . .	0.017	0.027	0.084	0.126	0.098	0.120
Length	0.400	1.296	2.250	3.300	3.000	3.000

In order to ascertain the comparative size of the tubules of *A. aegypti* with other mosquitoes the mature larvae of seven other genera were examined. It was found that the tubules of *A. aegypti* were larger than those of *Psorophora jamaicensis*, *Megarhinus portoricensis*, *Anopheles punctipennis*, *Culex quinquefasciatus*, *Urano- taenia sapphirina*, *Wyeomyia mitchelli* and *Mansonia perturbans*.

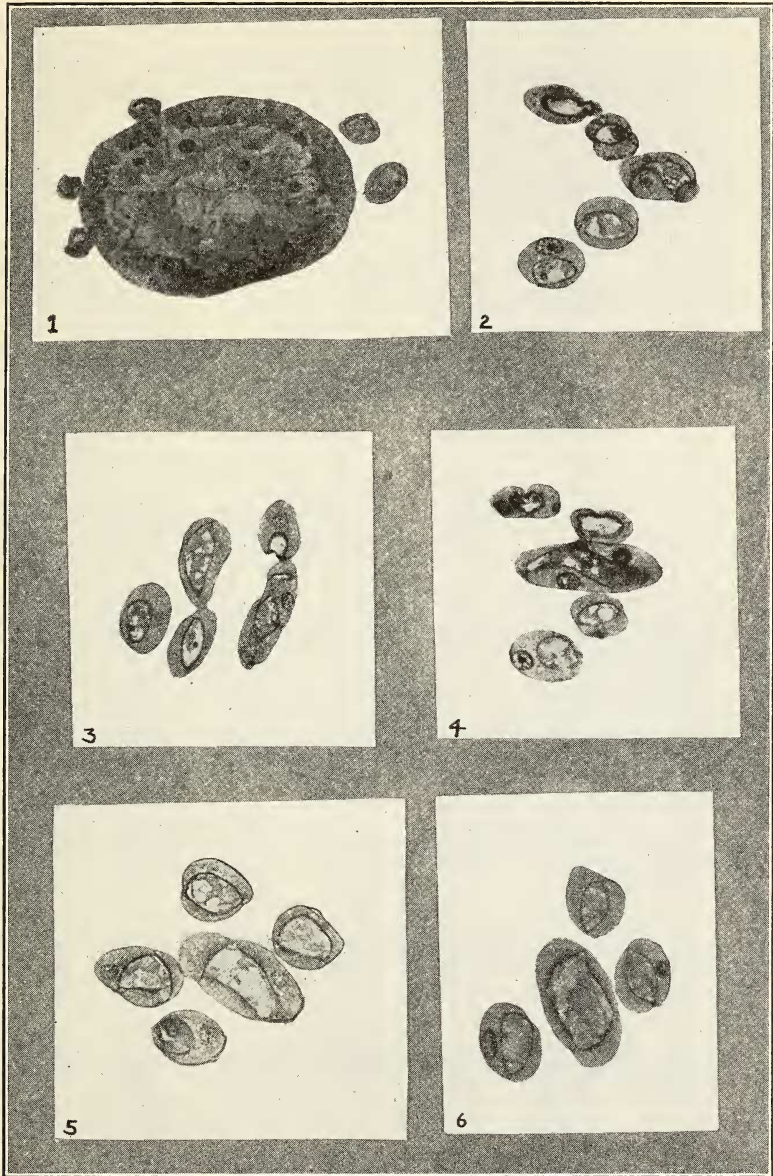
Whole mounts treated with osmic acid vapor and methyl green reveal the more darkly staining modified "internal border" which lines the portions of the cells facing the lumen of the tubules. Under this treatment also the nuclei are vesicular with large irregular dark staining central areas, and crystals resembling those of uric acid are numerous throughout the cytoplasm of the cells, especially in the distal portions of the tubules.

Sections of the fourth larval instar are useful in demonstrating clearly many of the features indicated in the living material and in the whole mounts. Sections made through the proximal regions of the tubules demonstrate their arrangement at their points of origin around the gut. Likewise, the flattened nature of the cells, the regular contour of the lumen and the lesser diameter of the tubule are apparent (Fig. 1). In the distal regions true cross sections show all gradations between a single cell entirely enveloping the lumen (Fig. 4) to two crescents joined at their tips (Fig. 2). Oblique sections may show portions of as many as four cells making up a tubule (Fig. 6). Such sections also demonstrate the tortuous course of the lumen caused by the bulging of the cells in their nuclear regions (Figs. 2, 4). Scattered throughout the cytoplasm of the cells are crystals resembling those of uric acid. These stain black with iron haematoxylin (Fig. 2) and exhibit spoke-like radiations from their centers. Golgi bodies appearing as small refractile spheres are found throughout the cytoplasm. A differentiated internal border which stains black with iron haematoxylin and blue with Mallory's triple stain is apparent throughout the entire lumen of the tubules (Figs. 1-6). This border appears to be a part of and a modification of the cytoplasm bordering the lumen. Occasionally, however, when adjacent cells are spread apart this internal border appears to be continuous. Under high magnifications this border appears irregularly striated.

The nuclei of these cells are large and vesicular and are characterized by a large irregular central body from which other chromatic material radiates in all planes (Fig. 2). In these respects the nuclei of the tubule cells are similar to those of the stomach (Fig. 1).

SUMMARY.

1. The five Malpighian tubules of *Aedes aegypti* L. arise anterior to the constriction between the stomach and intestine; four of these tubules arise laterally, two on each side and the fifth arises dorsally.
2. The portions of the cells of the tubules adjacent to the lumen



are modified to form an internal border.

3. Golgi bodies and uric acid crystals are present in the cytoplasm of the cells.

EXPLANATION OF FIGURES.

(All sections $\times 125$)

- Figure 1. Section through posterior region of stomach showing arrangement of tubules.
- Figure 2. Sections through tubules showing uric acid crystals within cytoplasm. Section to right demonstrates nuclear bulge (oblique section).
- Figure 3. Sections of tubules in oblique and transverse planes. One section (upper left) demonstrates a tracheole in sagittal section.
- Figure 4. Sections of tubules in oblique and transverse planes. One section (lower left) demonstrates continuity of internal border across gap at cell junction.
- Figure 5. Sections of tubules in oblique and transverse planes. One section (upper right) demonstrates basement membrane loosened from tubule.
- Figure 6. Sections of tubules in oblique and transverse planes. One section (center) shows four cells (oblique section).